Chapter 7

SCREENING WHITE-ROT FUNGI FOR THEIR CAPACITY TO DELIGNIFY WOOD

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I. INTRODUCTION

The higher fungi are the primary utilizers of complex sources of carbon in the terrestrial environment. The white-rot fungi, in particular, are known to decompose lignin, cellulose, and hemicellulose. Very little has been reported, however, on the screening of these fungi for their capacity to preferentially delignify wood. Kirk and Moore[1] examined nine selected white-rot fungi and found that seven of them lowered the lignin content of wood. Ander and Eriksson[2] screened 25 white-rot fungi on pine wood and kraft lignin as well as tested the influence of different nutrients on lignin degradation. *Pycnoporus cinnabarinus* Jacq. ex Fr. was found to degrade kraft lignin without loss of carbohydrates, but only if the weight loss was kept below 4%.

During the last decade, several researchers have suggested that ligninolytic fungi might be used in pulping operations (see Volume II, Chapter 14). Others have advocated the use of ligninolytic microorganisms to upgrade forest residues and other lignocellulosics for use as animal feed[3] (Volume II, Chapter 13). Both educational and private organizations have actively solicited industrial funds to explore the feasibility of such operations, and more recently, several laboratories have obtained federal funds to investigate microbial decomposition of lignin. At least one U.S. patent has been issued covering microbial pulping,[4] and developments in this area are being monitored by most of the major pulp and paper and forest products companies in the U.S. However, the patented process has not been commercialized, and there is little on-going industrial research in the U.S. to develop microbial pulping. The industrial skepticism arises mainly from the nonspecificity of many species toward lignin and the comparatively slow reported reaction rates of microbial delignification. These rates indicate that the industrial reactor capacity required for microbial pulping will be one to two orders of magnitude larger than that required in conventional chemical pulping operations.

In our preliminary investigations of microbial lignin degradation reported here, we have concentrated on screening white-rot fungi for their ability to decompose lignin in wood. The initial goals were to screen large numbers of fungi for their capacity to delignify wood, their growth rate, and for their phenol-oxidizing enzyme activity.

II. CULTURES

Cultures of white-rot fungi utilized in these studies were derived from various institutional collections or were newly collected in New York State or in the tropics. The known cultures from various collections were selected for rapid growth and for production of asexual spores in culture. The isolates collected in New York and the tropics are random collections of white-rot fungi, usually isolated from sporocarps found on white-rotted wood.

III. GROWTH STUDIES

Growth rates on malt agar were determined at 30 and 35°C for over 400 of the strains. As mentioned above, culture collection strains were chosen based on reports of rapid growth. A greater proportion of those strains other than of the newly isolated tropical strains (Puerto Rican and East African) grew rapidly at the two temperatures. Surprisingly, a higher proportion of the strains newly isolated in New York State other than tropical strains also grew rapidly at the relatively high test temperatures. Of the tropical strains, 73 to 90% grew slower than 0.5 cm/day; 20 failed to grow at 30 or 35°C at all and were not included in Table 1.

TABLE 1

Summary of Growth Rate Studies[a]

Source	Percentage of total strains					
	High growth rate > 1.0 cm/day		Medium growth rate 0.5—1.0 cm/day		Low growth rate < 0.5 cm/day	
	30°C	35°C	30°C	35°C	30°C	35°C
Culture collections (68)[b]	28	22	35	28	37	50
New York isolates (41)	34	27	42	17	24	56
Puerto Rico (134)	5	4	22	10	73	86
East Africa (147)	7	4	18	6	75	90

[a] Growth was assessed in petri dishes containing 1.5% malt extract, 0.1% yeast extract, and 1.5% Bacto agar medium. Average radial growth was measured at the end of 1 and 2 weeks.

[b] Number in parentheses refers to total number of tested strains.

Comparison of the individual growth data showed that most (68 to 87%) of the strains grew better at 30 than 35°C. Of the newly isolated strains, 10% from the tropics grew better at 35°C, whereas only one strain (2%) from New York did. Surprisingly, 14% of the strains from other culture collections grew better at 30 than 35°C. (None of these figures comparing growth at 30 and 35°C includes strains which overgrew the test plates at both temperatures during the 1-week growth tests. There were 9, 10, 1, and 2 such strains from the culture collection (known strains, New York, Puerto Rico, and East Africa sources, respectively.)

These results suggest that tropical white-rot fungi do not as a group grow more rapidly than temperate isolates at the relatively high test temperatures studied.

IV. DECAY STUDIES

In considering decay, the first question we addressed was how to screen fungi for the capacity to degrade lignin in wood. Important considerations included decay rate and fungal specificity. In Table 2, preliminary 5-week decay studies with 3 wood decay fungi and 1 soil-inhabiting mycoparasite (*Corticium* H) were conducted using agar block and soil block techniques. These results indicate that the three white-rot fungi decayed wood and degraded lignin in both cases. The small group of wood decay fungi tested decayed wood more rapidly in the soil block assay than in the agar block technique, and the soil isolate caused little change. The preliminary results suggest that delignification might be enhanced by the agar block method even though weight losses are much less than in the soil block test, except for *Corticium* H. Because of the more rapid decay rate, all further studies were conducted with the soil block technique.

In the next series of experiments, we extended the soil block procedure to a number of white-rot fungi from various culture collections. Results are summarized in Table 3. None of these organisms decayed wood as rapidly as *Phanerochaete chrysosporium* (approximately 5% per week). *Polyporus anceps, Poria latemarginata,* and *Corticium* B produced weight losses exceeding 4%/week, and *Tyromyces* C, *Poria subvermispora,* and *Coriolus hirsutus* caused weight losses exceeding 3%/week. In Table 3, we

TABLE 2

Comparison of Soil Block and Agar Block Decay Test Procedures[a]

Strain	Agar block[b]		Soil block[c]	
	Weight loss (%)[d]	Residual lignin (%)[e]	Weight loss (%)	Residual lignin (%)
Merulius tremellosus JHG-344	11.8	15.60	18.4	17.91
Phanerochaete chrysosporium ME-461	6.2	18.65	20.6	17.91
Phlebia radiata L-13688	2.6	17.48	6.2	18.55
Corticium H	3.6	19.48	1.8	20.34
Control	—	19.84	—	19.84

[a] All tests were conducted at 30°C and 70% relative humidity and employed the outer clear sapwood of *Populus grandidentata.*

[b] In the agar block method,[5] sterile cross section wafers, 2.5 × 2.5 × 1 cm, were used. After an initial 10-day incubation, wafers were transferred to fresh plates which were then incubated in plastic bags to prevent drying.

[c] Soil block tests were conducted with the 2.5 × 2.5 × 1 cm or 1.5 × 2.0 × 0.03 cm cross section wafers. The standard ASTM method[6] was used.

[d] Weight losses are based on original and final oven dry weights and expressed as a percentage of the former.

[e] Lignin was determined by the 72% H_2SO_4 method.[7]

have included an indication of preferential lignin utilization, "specificity". Specificity is the ratio:

$$\frac{\text{lignin consumed (g)}}{\text{nonlignin components consumed (g)}}$$

Since the aspen wood contains 20% lignin, a specificity of 0.25 means that lignin is consumed in a nonpreferential manner. Values greater than 0.25 indicate that the decayed wood is enriched in nonlignin components, whereas values less than 0.25 indicate that the decayed wood is enriched in lignin. The specificity of lignin removal increased or decreased in time with equal frequency. *Poria rivulosa* and *Punctularia strigosozonata* initially removed more lignin that nonlignin components from the decaying wood, whereas other fungi such as *Poria latemarginata, Polyporus occidentalis, Cerrena unicolor, Coriolus hirsutus, Tyromyces* C, and *Panus rudis* showed a preference for the carbohydrate portion of the wood after 4 and/or 8 weeks. Several strains showed relatively high specificities toward lignin (>0.4). The faster-growing strains decayed wood more rapidly than the slower-growing strains in the test, although this correlation was not observed in any of the later tests with other strains.

Additional strains from culture collections were examined in a separate test (Table 4) in which a slightly modified soil block procedure was used. None of the strains showed outstanding specificity for lignin. In this test, no correlation was seen between decay rate and growth rate on malt agar. Several strains decayed wood as fast or faster than *Phanerochaete chrysosporium,* and one of these, *Poria cinerascens* HHB-105, exhibited higher specificity toward the lignin.

Soil block decay studies were continued with newly isolated temperate strains (from New York), using both the standard and modified (as above) soil block procedures

TABLE 3

Decay Studies With Strains From Culture Collections Using Standard Soil Block Procedure[a]

Strain	Duration (weeks)	Weight loss (%)	Residual Lignin (%)	Specificity[b]	Growth rate[c]
Bjerkandera adusta FP-105256	4	5.16	19.05	—	H
	8	10.32	19.57	0.29	
Cerrena unicolor FP-125033	4	7.92	19.73	—	L
	8	16.83	21.72	0.12	
Coriolus hirsutus RLG-5133	4	10.07	20.54	0.16	H
	8	35.47	19.89	0.25	
Corticium B MS-53	4	12.80	18.36	0.43	H
	8	41.47	11.73	0.46	
Panus rudis GAL-123 sp	4	5.70	19.91	—	M
	8	22.16	21.34	0.17	
Peniophora mutata FP-104324	4	7.94	19.90	—	M
	8	13.13	20.01	0.24	
Phanerochaete chrysosporium ME-461	5	20.6	18.27	0.35	H
	5	19.8	19.08	0.30	
	8	48.8	18.82	0.27	
Pholiota adiposa OKM-4958	4	4.84	20.24	—	L
	8	5.02	18.55	—	
Polyporus anceps JRH- 82	4	16.16	17.86	0.44	H
	8	39.78	11.47	0.48	
Polyporus berkeleyi FP-105839	4	1.93	19.03	—	L
	8	10.23	18.49	0.47	
Polyporus occidentalis LCF-582	4	8.00	20.32	—	H
	8	33.42	20.44	0.23	
Poria latemarginata FP-104044	4	17.45	21.66	0.13	H
	8	33.78	23.23	0.15	
Poria rivulosa L-10602	4	6.15	17.11	—	H
	8	12.75	17.34	0.59	
Poria subvermispora FP-104027	4	11.12	19.32	0.32	H
	8	39.09	13.29	0.43	

TABLE 3 (continued)

Decay Studies With Strains From Culture Collections Using Standard Soil Block Procedure[a]

Strain	Duration (weeks)	Weight loss (%)	Residual Lignin (%)	Specificity[b]	Growth rate[c]
Poria subvermispora L-6332	4	8.52	18.43	—	H
	8	27.09	15.23	0.48	
Poria vincta TPRI 156	4	0	19.65	—	L
	8	3.30	20.28	—	
Punctularia strigosozonata PL-261	4	7.13	17.30	—	L
	8	8.23	15.22	—	
Trametes ravida FRI-395	4	7.04	19.44	—	L
	8	31.17	20.90	0.21	
Tyromyces C	4	19.10	20.34	0.22	L
	8	23.86	21.45	0.17	

[a] Procedures given in Table 2.

[b] Specificity = (lignin consumed [g])/(nonlignin components consumed [g]); specificities were not calculated where weight losses were less than 10%.

[c] Growth at 30°C (Table 1). H (high), > 1 cm/day; M (medium), 0.5 to 1.0 cm/day; L (low), < 0.5 cm/day.

TABLE 4

Decay Studies With Strains From Culture Collections Using A Modified Soil Block Procedure[a]

Strain	Duration (weeks)	Weight loss (%)	Residual lignin (%)	Specificity[b]	Growth rate[c]
Daedalea elegans ALW-3097	4	7.43	20.51	0.14	H
	8	19.22	19.62	0.27	
Fomes fraxinophilus RLG-3738	4	14.32	18.28	0.16	M
	8	49.55	14.08	0.27	
Fomitopsis ulmaria FP-103737	4	7.16	20.52	0.13	—[d]
	8	19.74	18.98	0.31	
Inonotus hispidus FP-48229	4	2.81	19.52	—	L
	8	53.56	21.11	0.23	
Panus rudis GAL-123	4	8.22	20.42	0.16	M
	8	45.81	21.44	0.22	
Peniophora creamea MJL-66	4	7.68	20.25	0.18	H
	8	35.36	13.80	0.45	
Pererriporia subacida FP-94457	4	1.30	20.21	—	L
	8	5.30	21.93	0	
Phellinus robustus FP-105694	4	5.31	18.70	0.69	L
	8	44.37	20.12	0.24	
Polyporus frondosus OKM-4954	4	2.16	20.29	—	—
	8	7.36	19.71	0.28	
P. graveolens L-15395	4	19.79	21.76	0.14	L
	8	50.78	24.54	0.18	
P. maximus ARS-58-26	4	19.62	19.02	0.30	H
	8	40.00	20.12	0.24	
P. supinus FP-90182	4	10.52	19.55	0.29	L
	8	40.59	19.76	0.25	
P. tulipiferae ARS-235	4	18.34	20.33	0.22	—[d]
	8	26.56	20.12	0.24	
Poria cinerascens FP-105273	4	3.30	19.47	—	H
	8	21.07	18.56	0.33	
P. cinerascens HHB-105	4	19.36	17.90	0.38	H
	8	55.73	11.96	0.35	

TABLE 4 (continued)

Decay Studies With Strains From Culture Collections Using A Modified Soil Block Procedure[a]

Strain	Duration (weeks)	Weight loss (%)	Residual lignin (%)	Specificity[b]	Growth rate[c]
Pycnoporus cinnabarinus FP-104138	4	11.32	19.90	0.25	M
	8	28.67	17.94	0.33	
P. coccineus FRI-154	4	24.05	16.69	0.43	M
	8	43.39	17.81	0.29	
P. sanguineus FP-103548	4	14.71	19.37	0.30	M
	8	32.65	19.20	0.27	
Spongipellis pachyodon FP-105866	4	13.55	18.89	0.36	M
	8	35.17	18.37	0.29	
S. spumeus var. *malicola* RLG-5622	4	3.31	20.22	—	M
	8	26.88	18.62	0.30	
Stereum frustulatum RZ-304	4	3.03	19.20	—	L
	8	11.10	19.01	0.37	
Trametes cingulata WY-2365	4	9.06	19.32	0.34	M
	8	27.66	19.99	0.24	
Vararia effuscata MJL-1059	4	15.37	17.95	0.44	M
	8	26.36	16.33	0.42	

[a] Standard procedure was modified by use of a filter paper feeder strip. Other procedures as in Table 2.

[b] Specificity = (lignin consumed [g])/(nonlignin components consumed [g]); specificities were not calculated where weight losses were less than 10%.

[c] Growth at 30°C (Table 1). H (high), > 1 cm/day; M (medium), 0.5 to 1.0 cm/day; L (low), < 0.5 cm/day.

[d] Not tested.

(Tables 5 and 6). (Four strains were tested with both procedures, and comparison shows clearly that higher weight losses are obtained with the standard test procedures than with this modification of it. Both tests are suitable for assessing specificity, however.) Three strains, ECS 1386, ECS 1363, and ECS 981, exhibited very marked specificity toward lignin, at weight losses of 11 to 16%. A strain of *Ischnoderma resinosus* showed no outstanding specificity, as had been reported with another strain of this species.[1] A number of the ECS strains decayed wood more rapidly and with lower specificity than *Phanerochaete chrysosporium* (Table 3). Other ECS strains decayed wood less rapidly than *P. chrysosporium*, but demonstrated equal or greater specificity for lignin. Several strains decayed wood less rapidly and with lower specificity than *P. chrysosporium*.

Decay tests with seven Puerto Rican isolates (Table 7) disclosed three which exhibited impressive specificities: ECS 1140b, ECS 1153, and especially ECS 1240. These have not yet been identified. The last two were shown to grow rapidly at both 30 and 35°C. None of the Puerto Rican isolates decayed wood as rapidly as *P. chrysosporium* (Table 4), although this could have reflected the different decay test procedures.

Decay studies with East African isolates are summarized in Table 8. Several of these strains decayed wood far more rapidly than any strains from other sources, including *P. chrysosporium*. Again, however, this might reflect in part the differences in test procedures used. With the East African isolates, we used a Highley-Scheffer modification which has been reported[8] to enhance decay rates. Outstanding specificity toward lignin was observed with LDT strains 9, 123, 287, 289, 296, 466, 594, and 682. Strains 9, 287, and 682, in fact, removed (Klason) lignin on a weight basis as rapidly as nonlignin components during the first 10 to 16% loss in total weight. Strains 9, 123, 287, and 289 grew slowly (<0.5 cm/day) at 30°C, whereas strains 296, 466, 594, and 682 exhibited moderate growth rates (0.5 to 1.0 cm/day) on malt agar plates.

V. PHENOL-OXIDIZING ENZYMES AND EXTRACTIVES

Phenol-oxidizing (PO) enzyme activity was assessed[9,10] with 46 of the newly isolated strains from New York and Puerto Rico. Fourteen of the isolates exhibited no PO activity (no coloration with various phenols), and the others gave varying color reactions with different phenolic substrates. No relationship could be discerned between the PO activity and growth rates, decay rates, or specificity. Similar results were obtained with 34 East African isolates.

Using the modified soil block technique, we examined the content of extractives (benzenehanol, 2:1, v/v, solubles) during decay by 62 strains. Results indicated a tendency for extractives to increase with decay, up to 10 to 12% on a decayed weight basis in some cases. However, no relationship was observed between extractives content and specificity toward lignin.

We have made an attempt to relate growth characteristics and phenol-oxidizing enzyme reactions of the African isolates to their rates and specificities of wood decay. In Table 9, we have summarized these data for all East African isolates on which we have complete data. The decay studies were conducted simultaneously under identical conditions. Seven cultures exhibited strongly positive oxidase reactions and decay rates from less than 0.5%/day with LDT-682 to well over 1.0%/day with LDT-857. The specificities of these isolates toward lignin ranged from low for LDT-617 to high for LDT-682. Likewise, those isolates with moderately positive oxidase reactions exhibited a similar range of growth rates, decay rates, and specificities. White-rot fungi with weakly positive and negative oxidase reactions also exhibited a full range of decay rates and lignin specificites. The results are similar to those of Kirk and Kelman[11] who found

TABLE 5

Soil Block Studies With Temperate Strains Using The Standard Test Procedure[a]

Strain	Duration (weeks)	Weight loss (%)	Residual lignin (%)	Specificity[b]	Growth rate[c]
ECS-904	5	20.24	18.73	0.32	H
	8	26.09	18.82	0.30	
ECS-924	5	24.18	19.44	0.27	H
(*Polyporus brumalis*)	8	38.44	16.73	0.33	
ECS-925	5	37.47	21.25	0.21	H
	8	39.76	21.23	0.22	
ECS-967	5	38.19	21.12	0.22	M
(*Coriolus versicolor*)	8	49.38	22.62	0.21	
ECS-969	5	39.50	18.85	0.27	M
(*Coriolus versicolor*)	8	52.17	21.83	0.22	
ECS-970	5	15.55	18.72	0.35	H
(*Daedalea confragosa*)	8	33.27	21.08	0.21	
ECS-971	5	20.27	20.78	0.19	H
(*Bjerkandera adusta*)	8	31.44	21.37	0.20	
ECS-981	5	12.90	19.93	0.24	M
(*Polyporus dichrous*)	8	16.07	15.53	0.74	
ECS-986	5	23.16	17.78	0.36	M
(*Phlebia radiata*)	8	29.18	19.18	0.27	
ECS-998	5	34.80	21.23	0.21	M
(*Irpex tulipiferae*)	8	53.57	22.42	0.12	
ECS-1354	5	20.69	20.18	0.23	H
(*Hericium coralloides*)	8	28.32	21.14	0.20	
ECS-1355	4	29.22	20.34	0.23	H
	8	55.52	21.79	0.22	
ECS-1358	4	31.42	19.72	0.25	H
	8	45.98	21.94	0.21	
ECS-1388	4	30.73	20.61	0.22	H
(*Phyllotopsis nidulans*)	8	45.62	21.60	0.22	
ECS-1398	4	29.56	20.40	0.23	H
	8	65.66	20.29	0.24	

[a] Procedures Table 2.

[b] Specificity = (lignin consumed [g]/(nonlignin components consumed [g]); specificities were not calculated where weight losses were less than 10%.

[c] Growth at 30°C (Table 1). H (high, > 1 cm/day; M (medium), 0.5 to 1.0 cm/day; L (low), < 0.5 cm/day.

that the inability of certain wood decay fungi to oxidize phenols cannot be assumed to indicate inability to utilize lignin.

VI. DISCUSSION AND CONCLUSIONS

Although some tropical white-rot fungi grow more rapidly at 35 than at 30°C most grow better at the lower temperature. Of those that do grow better at 35°C, the difference in growth rates at the two temperatures is not great. Based on our results with isolates derived from various collections and with isolates collected in New York, rapidly growing microorganisms may be as available in the temperate regions as in the tropics.

We do not presently know how to simply select microorganisms that can preferentially utilize lignin in wood. Growth rate, phenol-oxidizing enzyme activity, and extractives production during decay could not be correlated with specificity toward lignin. The tedious test we employed was developed to determine the resistance of wood to fungal decay, and it encourages rapid consumption of wood. While the soil blocktest

TABLE 6

Soil Block Studies With Temperate Strains Using A Modified Soil Block Procedure[a]

Strain	Duration (weeks)	Weight loss (%)	Residual lignin (%)	Specificity[b]	Growth rate[c]
ECS-924	4	10.4	20.41	0.18	H
(*Polyporus brumalis*)	6	19.9	19.34	0.28	
ECS-930	4	2.7	17.70	—	M
(*Coriolus versicolor*)	6	3.8	17.49	—	
ECS-967	4	17.9	19.86	0.25	M
(*Coriolus versicolor*)	6	28.6	19.41	0.27	
ECS-968	4	3.9	18.29	—	M
(*Poria subacida*)	6	8.9	15.25	—	
ECS-969	4	10.0	21.01	0.11	M
(*C. versicolor*)	6	36.8	19.15	0.27	
ECS-971	4	2.6	20.36	—	H
(*Bjerkandera adusta*)	6	7.5	20.63	—	
ECS-972	4	5.9	16.72	—	M
(*B. adusta*)	6	8.4	15.70	—	
ECS-1363	4	3.4	19.85	—	L
(*Phlebia radiata*)	6	12.3	13.65	1.80	
ECS-1367	4	12.3	19.07	0.25	M
C. versicolor)	6	24.5	17.24	0.39	
ECS-1374	4	1.8	17.37	—	M
(*Phlebia radiata*)	6	6.9	17.16	—	
ECS-1384	4	4.5	19.47	—	L
(*Ischnoderma resinosus*)	6	8.5	18.71	—	
ECS-1386	4	5.9	17.80	—	M
(*Phlebia radiata*	6	11.4	16.15	0.96	
ECS-1401	4	8.5	19.93	—	H
(*Polyporus brumalis*)	6	17.0	19.53	0.24	

[a] See footnote a, Table 4.

[b] Specificity = (lignin consumed [g])/(nonlignin components consumed [g]); specificities were not calculated where weight losses were less than 10%.

[c] Growth at 30°C (Table 1). H (high), > 1 cm/day; M (medium), 0.5 to 1.0 cm/day; L (low), < 0.5 cm/day.

was not ideally suited to our purpose, it did disclose several strains which preferentially utilize lignin. It showed also that there is variation within species in capacity to decay wood and in specificity for lignin, as might be expected.

Modifications of the soil block test resulted in substantial changes in rate of decay, and they may have caused marked changes in specificity of decay for lignin. The Highley-Scheffer[8] modification of the American Society for Testing and Materials (ASTM) technique, wherein a paper feeder strip and malt extract solution were added to the soil rather than water, was superior in terms of weight loss to tests where no or only small amounts of malt extract solution were added to the soil rather than water alone. Our results support their claim that their test for determining resistance of wood to decay is better for white-rot fungi than the ASTM technique. These results are in accord with Ander and Eriksson's finding[2] that malt extract was found generally to give the strongest lignin degradation. The decay rate also increased with the Highley-Scheffer modified ASTM technique and, in some cases, the time for the testing may be reduced to 2 weeks.

In general, the specificity of decay caused by an organism appears to increase to a maximum value between 20 and 40% weight loss and then decline. This maximum probably varies from organism to organism. The higher selectivity at lower weight losses suggests a greater accessibility of the initially removed lignin.

TABLE 7

Decay Studies With Puerto Rican isolates[a]

Strain	Duration (weeks)	Weight loss (%)	Residual lignin (%)	Specificity[b]	Growth rate[c]
ECS-1010	4	10.9	19.40	0.31	M
(*Trametes corrugata*)	6	14.9	18.51	0.38	
ECS-1140b	4	11.6	17.04	0.71	—
	6	21.1	15.24	0.59	
ECS-1147	4	5.9	20.99	—	H
	6	6.4	21.25	—	
ECS-1151	4	8.2	18.11	—	M
	6	9.6	17.44	—	
ECS-1153	4	17.4	17.16	0.49	M
	6	24.2	17.61	0.37	
ECS-1190	4	6.2	17.23	—	M
	6	8.3	14.13	—	
ECS-1193	4	2.9	18.88	—	—
	6	3.0	17.82	—	—
ECS-1240	4	12.3	15.89	0.92	—
	6	22.8	12.86	0.77	—

[a] Modified soil block procedure. Other procdures given in Table 2.

[b] Specificity = (lignin consumed [g])/(nonlignin components consumed [g]); specificities were not calculated where weight losses were less than 10%.

[c] Growth at 30°C (Table 1). H (high), > 1 cm/day; M (medium), 0.5 to 1.0 cm/day; L (low), < 0.5 cm/day.

TABLE 8

Decay Studies With East African Isolates[a]

Strain	Duration (weeks)	Weight loss (%)	Residual lignin (%)	Specificity[b]	Growth rate[c]
LDT-2	3	11.6	20.27	0.20	M
	6	15.1	19.44	0.29	
LDT-9	3	6.2	15.70	—	L
	6	16.6	13.85	1.01	
LDT-14	3	40.0	17.40	0.31	M
	6	57.8	14.87	0.31	
LDT-52	3	24.5	18.49	0.32	M
	6	51.1	20.43	0.24	
LDT-56	3	36.2	19.98	0.25	H
	6	41.6	21.35	0.22	
LDT-102	3	57.5	20.48	0.24	H
	6	78.6	24.64	0.23	
LDT-123	3	21.3	16.33	0.49	L
	6	48.2	18.46	0.27	
LDT-287	3	11.3	15.89	1.05	L
	6	30.3	11.19	0.66	
LDT-289	3	9.9	17.03	—	L
	6	22.3	14.11	0.67	
LDT-295	3	13.6	18.74	0.37	M
	6	33.2	19.23	0.27	
LDT-296	3	12.4	17.90	0.51	M
	6	28.0	16.12	0.42	
LDT-315	3	33.3	19.76	0.25	M
	6	71.6	19.68	0.25	
LDT-320	3	42.6	22.94	0.19	H
	6	62.4	22.09	0.23	
LDT-334	3	30.9	22.15	0.17	M
	6	53.4	23.94	0.20	

TABLE 8 (continued)

Decay Studies With East African Isolates[a]

Strain	Duration (weeks)	Weight loss (%)	Residual lignin (%)	Specificity[b]	Growth rate[c]
LDT-393	3	6.3	19.66	—	—
	6	8.5	20.06	—	
LDT-402	3	20.7	18.17	0.36	—
	6	42.9	18.86	0.27	
LDT-405	3	50.8	21.86	0.22	H
	6	73.4	25.74	0.22	
LDT407	3	50.7	19.61	0.25	H
	6	61.2	20.32	0.24	
LDT-409	3	50.7	22.08	0.22	L
	6	72.3	27.70	0.20	
LDT-417	3	13.1	18.73	0.38	M
	6	27.8	20.01	0.24	
LDT-424	3	69.3	21.22	0.24	H
	6	88.6	21.12	0.24	
LDT-463	3	6.6	18.58	—	L
	6	15.3	20.12	0.23	
LDT-466	3	16.0	17.16	0.52	M
	6	36.0	18.10	0.30	
LDT-586	3	9.2	16.52	—	L
	6	28.5	20.01	0.24	
LDT-594	3	38.0	13.91	0.42	M
	6	50.5	18.84	0.26	
LDT-610	3	15.0	18.52	0.38	M
	6	46.3	15.01	0.34	
LDT-617	3	39.9	21.62	0.21	H
	6	55.1	22.36	0.22	
LDT-623	3	11.2	20.33	0.20	M
	6	18.9	20.62	0.20	
LDT-655	3	12.4	18.74	0.39	M
	5	25.0	13.91	0.65	
LDT-657	3	59.0	22.00	0.23	H
	6	77.2	23.23	0.23	
LDT-665	3	58.4	20.65	0.24	M
	6	85.5	23.93	0.24	
LDT-682	3	10.4	15.24	1.49	M
	6	28.2	12.61	0.62	
LDT-684	3	26.8	15.21	0.48	L
	6	54.4	17.84	0.26	
LDT-752	3	6.2	20.14	—	L
	6	21.7	21.86	0.15	
LDT-857	3	15.4	18.45	0.38	L
	6	32.8	20.35	0.23	
LDT-881	3	12.4	20.00	0.24	M
	6	25.3	20.01	0.24	
LDT-1005	3	34.1	16.46	0.36	M
	5	51.8	15.37	0.32	
LDT-1033	3	23.2	18.33	0.33	H
	6	59.8	20.30	0.25	
LDT-1182	3	46.5	18.40	0.28	L
	6	54.3	18.73	0.26	

[a] Modified soil block procedure. Other procedures given in Table 2.

[b] Specificity = (lignin consumed [g])/(nonlignin components consumed [g]); specificities were not calculated where weight losses were less than 10%.

[c] Growth at 30°C (Table 1). H (high), > 1 cm/day; M (medium), 0.5 to 1.0 cm/day; L (low), < 0.5 cm/day.

TABLE 9

Summary of Studies With East African Isolates

Isolate	Phenol-oxidizing enzyme reactions[a]	Growth rate[b]	Decay rate	Specificity[c]
LDT-52	Strong	Moderate	Rapid	0.24—0.32
LDT-56	Strong	Moderate	Rapid	0.22—0.25
LDT-295	Strong	Moderate	Moderate	0.27—0.37
LDT-296	Strong	Moderate	Moderate	0.42—0.51
LDT-617	Strong	Rapid	Rapid	0.21—0.22
LDT-682	Strong	Moderate	Slow	0.62—1.49
LDT-857	Strong	Moderate	Moderate	0.23—0.38
LDT-14	Moderate	Moderate	Moderate	0.31
LDT-463	Moderate	Moderate	Slow	0.23
LDT-586	Moderate	Slow	Moderate	0.24—1.13
LDT-594	Moderate	Moderate	Rapid	0.26—0.42
LDT-623	Moderate	Moderate	Moderate	0.20
LDT-684	Moderate	Moderate	Rapid	0.26—0.48
LDT-881	Moderate	Moderate	Moderate	0.24
LDT-2	Weak	Moderate	Moderate	0.20—0.29
LDT-102	Weak	Rapid	Rapid	0.23—0.24
LDT-123	Weak	Moderate	Rapid	0.27—0.49
LDT-289	Weak	Moderate	Slow	0.67
LDT-315	Weak	Moderate	Rapid	0.25
LDT-334	Weak	Moderate	Rapid	0.17—0.20
LDT-405	Weak	Rapid	Rapid	0.22
LDT-417	Weak	Moderate	Moderate	0.24—0.38
LDT-610	Weak	Moderate	Moderate	0.34—0.38
LDT-657	Weak	Rapid	Rapid	0.23
LDT-665	Weak	Moderate	Rapid	0.24
LDT-1005	Weak	Moderate	Rapid	0.32—0.36
LDT-1033	Weak	Rapid	Rapid	0.25—0.33
LDT-9	Negative	Moderate	Slow	1.01
LDT-102	Negative	Rapid	Rapid	0.23—0.24
LDT-287	Negative	Moderate	Moderate	0.66-1.05
LDT-407	Negative	Rapid	Rapid	0.24—0.25
LDT-409	Negative	Slow	Rapid	0.20—0.22
LDT-424	Negative	Rapid	Rapid	0.24
LDT-1182	Negative	Moderate	Rapid	0.26—0.28

[a] Based on color reactions with phenolic substrates.[8,9]

[b] See Table 1.

[c] Specificity = (lignin consumed [g])/(nonlignin components consumed [g]); specificities were not calculated where weight losses were less than 10%.

A number of strains studied here, most notably *Corticium* B (Table 7-3), *Phellinus robustus* FP-105694, *Pycnoporus coccineus* FRI-154, *Vararia effuscata* MJL-1059 (Table 4), ECS isolates 1140b, 1153 and 1240 (Table 7); and LDT isolates 123, 287, 296, 466, 594, and 682 (Table 8); showed marked specificity toward lignin during 3- to 4-week decay tests. They should be examined more closely. The results clearly show that nature offers a wide variation in specificities of white-rot fungi and suggest that tropical sites, as hoped, are a rich source of highly ligninolytic strains.

With present technology, we do not envision specific industrial applications for microbial decomposition of lignocellulosics. Conceptually, microbial delignification could be used (1) as a substitute for one or more of the chemical pulping and bleaching operations, (2) as a pretreatment in chemical or mechanical pulping, (3) as a means to

upgrade unutilized and underutilized agricultural residues for animal feeds, and (4) as a process to overcome the lignin barriers to enzymatic hydrolysis of cellulose to glucose. Ligninolytic microorganisms might also be employed (1) to transform lignocellulosics and chemically modified lignins into useful noncarbohydrate specialty or commodity chemicals, (2 to remove soluble lignin residues from industrial waste streams, (3) to control or improve soil fertility, and (4) to manipulate the flux rate and magnitude of the organic component of soil, one of the larger organic carbon reservoirs. Before any of these potential applications can be reduced to practice, we need to know more about the microorganisms that decompose lignin and how they can be manipulated to accomplish a series of different tasks. Although applications of ligninolytic microorganisms are likely to occur before we have a complete understanding of lignin catabolism, research in this area will allow rapid development and application of improved processes for pulping and for utilization of our unutilized lignocellulose resource.

VII. SUMMARY

Strains of white-rot fungi from culture collections and newly isolated in New York State and in the tropics (Puerto Rico and East Africa) were examined for: (1) growth rates on malt agar at 30 and 35°C (more than 400 strains), (2) decay rates at 30°C in soil block tests with aspen wood (more than 100 strains), (3) specificities toward lignin during decay (more than 100 strains), and (4) PO enzyme activities (more than 400 strains). Most of the strains grew better at 30 than 35°C; the tropical fungi did not as a group grow more rapidly than temperate isolates at these temperatures. Decay rates varied markedly and were not correlated with growth rates on malt agar or with PO activity. Several strains, particularly from East Africa, were quite selective for lignin during decay. No relationship was found between specificity for lignin, decay rates, growth rates, PO activity, or extractives content of the decayed wood.

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